Report for 2003ND22B: Effects of West Nile Virus Infection, Immune Function, and Age on Female Yellow-headed Blackbird (Xanthocephalus xanthocephalus) Reproduction

- Other Publications:
  - Jennifer Newbrey and Wendy Reed, 2003,DETECTING WEST NILE VIRUS ANITBODIES IN CENTRAL NORTH DAKOTA YELLOW-HEADED BLACKBIRDS, Entomological Society of Manitoba meeting,Winnipeg, Manitoba, Canada.
  - Presentation: Jennifer Newbrey, Sept. 23, 2003, Effects of West Nile Virus Infection, Immune Function, and Age on Female Yellow-headed Blackbird (Xanthocephalus xanthocephalus) Reproduction,ND WRRI Advisory Committee Meeting, Bismarck, North Dakota.

Report Follows

EFFECTS OF WEST NILE VIRUS INFECTION, IMMUNE FUNCTION, AND AGE ON FEMALE YELLOW-HEADED BLACKBIRD (*XANTHOCEPHALUS XANTHOCEPHALUS*) REPRODUCTION

ND WRRI Graduate Research Fellowship Project
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DESCRIPTION OF THE CRITICAL WATER PROBLEM

Recent high water levels and canalization of water resources (i.e., Garrison diversion) in North Dakota have resulted in an increase of aquatic habitats for many wildlife species. Current water conditions correspond with increased numbers of wetland breeding birds and increased habitat for breeding mosquitoes. Because birds often serve as intermediate hosts for mosquito borne diseases, increased populations of bird and mosquitoes could impact the ecology, rate of emergence, and persistence of diseases in humans and wildlife. The recent spread of WNV into the state has produced a need for research to study the influence of the virus on North Dakota wildlife.

The North Dakota Department of Health reported the first cases of WNV in the state in the summer of 2002. The first bird to test positive for WNV was a crow found on July 14th, and the first positive human cases were reported on August 28th. Because stagnant water in wetlands is ideal breeding habitat for mosquitoes, wildlife associated with these habitats may suffer high rates of WNV infection. The recent arrival of WNV into the state necessitates a study of the prevalence and immunological impact of WNV on native North Dakota wetland species. Most research on the virus has focused on using carcasses of birds as a surveillance system for detecting the spread of WNV across North America. No published research has been conducted on a living population of free-ranging birds. Failure of biologists to adequately address disease emergence in free-ranging wildlife may lead to diminished geographic distributions and populations declines (Friend et al. 2001).

The prairie couteau region of central North Dakota has many small prairie wetlands, which provide essential foraging and breeding habitat for many species of birds. Yellow-headed blackbirds are an ideal species to study WNV infection because they breed in high-density wetland colonies, which insures a large sample size. Establishing rates of WNV infection in yellow-headed blackbirds is necessary to determine the vulnerability of this wetland dwelling species. Information gathered on WNV for this study can also be used to model and predict potential impacts of the virus on other species of wetland birds.

KEY LITERATURE

West Nile virus is a mosquito-borne virus that was first diagnosed in North America in 1999 (Rappole et al. 2000). Since that time, the virus has spread across the United States and into Canada. Because the virus can cause fatal meningitis, it has become a national health concern for human populations, an economic concern for domestic animal losses, and a concern for the status of free-living bird populations (Campbell et al. 2002). Birds are one of the principal hosts of WNV (Rappole et al. 2000), with more than 111 species of wild birds diagnosed with the virus in the United States since 1999 (CDC unpub. data). Most of the information we have on the prevalence and distribution of the virus is based on information from symptomatic birds (i.e., sick or dead), which can bias estimates of potential outbreaks if asymptomatic birds have survived infection or act as carriers of the virus. By testing for the presence of antibodies to WNV in yellow-headed blackbirds, I will be able to assess the degree to which a free-living population has been exposed to the virus, the degree to which the population can avoid an epidemic, and the potential for blackbirds to serve as carriers for WNV.

When a bird suffers immune system stress, resources are allocated away from nonessential processes, such as growth and reproduction, and are reallocated to activities directly related to survival (Lochmiller and Deerenberg 2000). For example, depressed female immunity is known to decrease the diversity and concentration of carotenoids in egg yolks (Saino et al. 2002). Carotenoids are biologically active, lipid-soluble pigments synthesized by plants and photosynthetic microorganisms, which animals must obtain from their diet (Blount et al. 2000). In developing avian embryos, carotenoids in the yolk protect vulnerable tissues against damage caused by free radicals, by-products of normal metabolism and immune defense, which can cause extensive DNA, protein, and lipid damage (Surai et al. 2001). Because carotenoids are derived solely from the female, they reflect the quality of the maternal diet prior to egg laying.

As powerful antioxidants and immunostimulants, carotenoids are also incorporated into the sexual signals of many animals and are thought to indicate individual health (Blount et al. 2000). Yellow-headed blackbirds depend on carotenoids for their brightly colored yellow plumage. Second year (SY) female yellow-headed blackbirds can be distinguished from older, after second year (ASY) females because they have smaller patches of yellow feathers and are paler in their head, neck and breast regions (Crawford and Hohman 1978). These differences in plumage may reflect a female's ability to obtain carotenoids from surrounding habitats and therefore reflect her ability to deposit carotenoids into her eggs (Royle et al. 2001). Female age could also have substantial impacts on resource allocation and immune function. Young females may allocate more physiological resources to growth than mature females, and therefore allocate less to reproduction and immunity.

Maternal tradeoffs exist between the use of carotenoids for physiological functions, the expression of sexual signals, and investment into eggs (Saino et al. 2002). Yellow-headed blackbirds are an ideal species to study maternal tradeoffs between reproduction and self-maintenance, because females can easily be separated into 2 age classes and they allocate carotenoids both to plumage and eggs. Also, the high density of conspicuous, easily accessible nests in small North Dakota wetlands makes yellow-headed blackbirds an ideal species for study of WNV infection, immune response, and carotenoid concentrations in breeding females and their offspring.

SCOPE AND OBJECTIVES

The overall objective of this project is to determine the effects of female age and infection with West Nile virus on yellow-headed blackbird (*Xanthocephalus xanthocephalus*) maternal investment into eggs. The specific objectives of this project are to identify the prevalence of WNV in a free-living population of yellow-headed blackbirds, to quantify variation in immune function of female blackbirds, and to measure the relationship between female immune function and age on carotenoid allocation to eggs. These objectives will allow us to evaluate potential relations between wetland bird WNV infection and increased aquatic habitat for breeding mosquitoes in North Dakota.

METHODS, PROCEDURES, AND FACILITIES

Female yellow-headed blackbirds will be captured using mist-nets in order to collect blood samples to assess WNV antibody production and to measure variation in immune function using non-lethal immune challenges. Blood serum will be tested for WNV antibodies using competitive enzyme-linked immunoabsorbent assay (ELISA). We will follow protocol specifically designed to detect WNV antibodies in blood serum from avian species (Dr. Barry Beaty, Colorado State University, pers. comm.). We will quantify variation in female immune function at both the cell-mediated (i.e., white blood cell) and humoral (i.e., antibody production) levels using the methods described in Casto et al (2001). To assess cell-mediated immunity, we will measure differential-cutaneous swelling between wings of 30 females injected with a harmless plant protein (phytohemaglutinin - PHA) in the right wing and saline solution in the left. We will assess variation in humoral immune response by quantifying the antibody production of 30 females injected with sheep red blood cells (a novel, non-lethal antigen). Prior to release, each female will be categorized as either SY or ASY and will be banded with a standard Fish and Wildlife Service aluminum band along with a unique color-band combination for individual field identification.

We will locate and monitor nests of marked females to assess maternally allocated carotenoids in eggs and offspring survival and growth. The third-laid egg will be removed from each nest for carotenoid analysis, which insures the detection of differences among females and not variation in carotenoid allocation due to egg laying order. Yolks will be separated from the egg and the carotenoids will be extracted from the yolk using the methods described in Surai and Speake (1998). We will analyze carotenoids with High Performance Liquid Chromatography (HPLC) using a reverse-phase column. We will assess the concentration of carotenoids by comparing my samples to a calibration curve obtained from known concentrations of carotenoids. After sampling the third egg, we will monitor each nest at 3-day intervals to determine hatching success and nestling growth rates (i.e., body mass, tarsus length, and wing length).

This study will be conducted on several wetlands located within a 20 square mile area of the prairie couteau region of central North Dakota (Stutsman County). Central North Dakota has one of the highest concentrations of yellow-headed blackbirds in North America (Twedt and

Crawford 1995). In addition, Stutsman County has one of the highest numbers of positive avian West Nile Virus cases in North Dakota (CDC unpub. data). Conducting this study in a high-density population of yellow-headed blackbirds with known exposure to WNV insures a large enough sample size for all analyses.

Dr. Wendy Reed will provide field facilities in Stutsman County for all field data collection. Lab analysis of WNV antibodies and carotenoid concentrations will be conducted at NSDU.

ANTICIPATED RESULTS AND DELIVERABLES

This study will provide essential information on the prevalence and immunological impact of WNV on a North American avian species. Infection with the virus can be lethal, however, the degree to which birds are adversely affected varies across species and even between individuals within a species (Rappole et al. 2000). By testing for the presence of antibodies to WNV in yellow-headed blackbirds, we will be able to assess the vulnerability and degree of virus exposure in a free-living population of wetland dwelling birds. We will also be able to evaluate potential influences of current high-water conditions on breeding populations of mosquitoes and avian WNV infection rates.

Many wildlife pathogens cause non-lethal physiological and reproductive effects that remain poorly understood. This study will quantify the immunological costs and maternal tradeoffs associated with exposure to a non-lethal antigen. Because female birds allocate essential resources to eggs, exposure to pathogens can shift maternal resources away from reproduction. This seemingly small, non-lethal effect influences the survival of offspring and can therefore cause population level effects in the next generation.

The research conducted for this study will comprise a Ph.D. dissertation. Results will be presented at an American Ornithologist Union conference and will ultimately be submitted for publication to a prestigious peer-reviewed journal.

RESULTS AND CONCLUSIONS

This study is being conducted on several wetlands located within a 5 square mile area of the Missouri coteau region of central North Dakota. The general area is marked with a yellow dot on this slide. Central North Dakota has one of the highest concentrations of yellow-headed blackbirds in North America, as you can see from this distribution map. In addition, the study area has one of the highest numbers of positive avian WNV cases in central North Dakota.

Yellow-headed blackbirds are an ideal species to study maternal tradeoffs between reproduction, self-maintenance, and the influence of WNV for 3 reasons:

The first is that female yellow-heads allocate carotenoids both to their yellow plumage and egg yolks, which will allow me to study carotenoid allocation tradeoffs. Yellow-heads are also a good species because they nest in high-density colonies and have conspicuous, easily accessible nests. And the third reason is that yellow-headed blackbirds nest in stagnant-water wetlands, which are ideal breeding habitats for mosquitoes, making yellow-heads at high risk of WNV exposure.

Yellow-headed blackbird exposure to WNV is being detected by the presence of antibodies specific to the virus in the blood of free-living individuals. A small blood sample is collected from the brachial vein of captured birds for lab analysis. Blood has also been collected from 3 other species in the Icteridae family including: red-winged blackbirds, common grackles, and Western meadowlarks.

To determine differences in carotenoid concentrations in female yellow-headed blackbirds, we will measure carotenoid levels in eggs and feathers of each female. Three yellow feathers will be collected from the breast of each captured female and the third-laid egg will be collected from each study nest. Carotenoids will be extracted from feather and yolk samples in the lab and will be analyzed using chromatography techniques.

Prior to release each female is weighed, measured, and banded with a standard aluminum band and a unique color combination for individual field identification.

We will monitor nests of each female to determine nest success and nestling performance and how differences in WNV exposure and carotenoid levels influences their reproductive output.

In the lab, we are using a blocking ELISA to detect WNV antibodies.

Thus far, we have tested 38 yellow-headed blackbirds, 15 grackles, 2 red-winged blackbirds, and 1 Western meadowlark for WNV antibodies. Only 2 birds were positive, 1 red-winged blackbird and a Western meadowlark.

We were surprised that we did not find WNV antibodies in yellow-headed blackbirds and common grackles. The virus was definitely present in our study area because we were able to detect WNV antibodies in 2 other Icterid species.

We have 2 working hypotheses on what may be occurring:

The first is that WNV is lethal in free-living yellow-headed blackbirds and common grackles. In a recent study by Komar et al., experimentally infected common grackles were shown to have a 33% death rate within 4.5 days of being infected with WNV. Perhaps death rates are closer to 100% in less hospitable natural conditions and that is why we were unable to detect individuals with antibodies. We did not observe large numbers of dead birds, but perhaps infected individuals avoid the breeding colonies where most of our effort has been focused.

Our second hypothesis is that WNV infection rates are low in yellow-headed blackbirds and common grackles. If infection rates are low, perhaps we did not have a large enough sample size to detect WNV infected individuals. This seems unlikely since 1 out of 2 red-winged blackbirds and the only western meadowlark sampled had WNV antibodies. Since all sampled birds were collected within a 5 square mile area, it would seem infection rates were actually high in our study area.

In their study, Komar et al. did not observe any deaths in experimentally infected red-winged blackbirds, indicating that the species is able to survive WNV infection by producing antibodies.

We hope to experimentally infect yellow-headed blackbirds with WNV in captivity to better understand how the species responds to infection. We have collected eggs and feathers from 20 female yellow-heads to study carotenoids, but since they were not infected with WNV we will be unable to study non-lethal WNV effects. If WNV infection is lethal in yellow-headed blackbirds, we may include red-winged blackbirds in our carotenoid research to study non-lethal effects of the virus.

This research is very important because it will provide information on the impact of WNV infection on North American Icterids. No published research has been conducted on a living population of free-ranging birds. North American avian species may experience virus related populations declines if biologists do not act quickly to learn more about the virus. You can see from this slide that yellow-headed blackbird populations in our study area increased by greater than 1.5 percent per year from 1966 to 1996. We have a poor understanding of how WNV will influence future population trends in this and other Icterid species.

The loss of large numbers of yellow-headed blackbirds and common grackles due to WNV infection would have ecological ramifications in the food-web dynamics of the prairie wetlands of North Dakota. Eggs, young, and adult birds of both species are an invaluable food source to predatory birds and mammals.

In addition, WNV induced yellow-headed blackbird population crashes could result in a population explosion of red-winged blackbirds. As the most abundant songbird in North America, red-winged blackbirds cause extensive crop damage in North Dakota annually. Yellow-heads help to control red-winged blackbird numbers by excluding red-wing breeding pairs from their colonies and competing for natural food sources.

WNV induced lethality in different bird species can also influence human health. Those bird species that suffer high death rates associated with WNV have been found to have high viremia levels circulating in their blood streams. They also have the highest probability of passing the virus to a mosquito vector. We believe WNV could be lethal in yellow-headed blackbirds and common grackles because we were unable to detect WNV antibodies in the individuals that we sampled. If the virus is lethal infected individuals may also have high virema levels and act as virus reservoirs. Therefore, further study is needed to test for WNV antibodies and how the virus impacts these 2 species, wetland ecology, and human health. We are very intrigued by our findings and will continue to collect more information on the effects of WNV infection on the Icterids in our study area.

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